Original Article

The clinicopathological significance of BRI3BP in women with invasive breast cancer

Abrar I. Aljohani[1]^, Ieman A. Aljahdali[1], Ohud A. Alsalmi[1], Meshari A. Alsuwat[1], Abdulaziz A. Alsharif[1], Khalid J. Alzahrani[1], Batool S. Alsaleh[2], Ameen Nadheef[2], Turki S. Alqurashi[3]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia; [2]Department of Laboratory and Blood Bank, King Abdulaziz Specialist Hospital, Taif, Saudi Arabia; [3]Al-Lith University College, Umm Al-Qura University, Makkah, Saudi Arabia

Contributions: (I) Conception and design: AI Aljohani, IA Aljahdali, OA Alsalmi, MA Alsuwat, AA Alsharif, KJ Alzahrani, BS Alsaleh, A Nadheef, TS Alqurashi; (II) Administrative support: BS Alsaleh, A Nadheef; (III) Provision of study materials or patients: AI Aljohani, OA Alsalmi, BS Alsaleh, A Nadheef; (IV) Collection and assembly of data: AI Aljohani, IA Aljahdali, OA Alsalmi, MA Alsuwat, AA Alsharif, KJ Alzahrani, TS Alqurashi; (V) Data analysis and interpretation: AI Aljohani, OA Alsalmi, TS Alqurashi; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Abrar I. Aljohani, PhD. Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Airport Rd, Alhawiyah, Taif 21974, Saudi Arabia. Email: abrar.g@tu.edu.sa.

Background: Invasive breast cancer (BC) is a highly life-threatening disease affecting women world-wide. While its early identification may benefit the provision of more effective therapies, several BC-associated factors may influence BC patients' therapeutic outcomes. Therefore, identifying novel prognostic and therapeutic targets for invasive BC can help with accurate prognosis and therapy-related decisions. The BRI3 binding protein (*BRI3BP*) gene was found to be a principal gene in invasive BC cohorts using artificial neural network (ANN) techniques. Thus, this study aimed to evaluate the clinicopathological significance of BRI3BP at the transcriptomic and proteomic levels in invasive BC.

Methods: Two transcriptomic BC cohorts, the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; n=1,980) and The Cancer Genome Atlas (TCGA; n=854), were used to evaluate BRI3BP expression at the mRNA level. Formalin-fixed paraffin-embedded (FFPE) tissues from an invasive BC cohort (n=100) were also used to evaluate BRI3BP expression at the protein level via immunohistochemistry. The association between BRI3BP expression, clinicopathological characteristics, and patient outcomes was evaluated.

Results: In both METABRIC and TCGA cohorts, high expression of *BRI3BP* was significantly associated with aggressive tumor features such as high histological grade, large tumor size, and lymph vascular invasion (LVI) positivity. At the protein level, high BRI3BP expression was associated with high histological grade, hormone receptor negativity, high expression of Ki67, and poor outcome.

Conclusions: This study revealed the prognostic significance of BRI3BP in invasive BC patients. Further functional assessment is needed to confirm the biological role of BRI3BP in BC.

Keywords: Invasive breast cancer (invasive BC); BRI3 binding protein (BRI3BP); prognosis

Submitted Jun 30, 2024. Accepted for publication Nov 13, 2024. Published online Dec 27, 2024.
doi: 10.21037/tcr-24-1113
View this article at: https://dx.doi.org/10.21037/tcr-24-1113

^ ORCID: 0000-0003-2378-4234.

© AME Publishing Company.

Introduction

Breast cancer (BC) is the most prevalent cancer that occurs in women globally (1). In Saudi Arabia, BC cases are increasing, currently representing 28.7% of all cancers. Thus, BC is a serious condition that should be controlled (2). Invasive BC has been evaluated visually, but no approach could identify the primary molecular factors influencing patient survival thus far. However, innovative genomic, transcriptomic, and proteomic methods have identified key molecular drivers of invasive BC (3). In recent decades, patients with early-stage BC have had better outcomes due to improved diagnostic accuracy, targeted therapy, and early cancer identification. Despite significant improvements in outcomes, metastasis remains the most significant cause of BC-related death, impacting over 20% of BC patients (4). Little effort has been made to evaluate prognostic factors for BC patients in Saudi Arabia. Therefore, studying prognostic and predictive factors of BC in Saudi Arabia is essential for determining the risk of metastasis and guiding treatment decisions.

Previously, artificial neural network (ANN) methodology was used to identify principal genes in BC using two large transcriptomic cohorts of BC: The Cancer Genome Atlas (TCGA) (5) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (6). The BRI3 binding protein (*BRI3BP*) was found to be one of the top genes in invasive BC (7). *BRI3BP* may have an impact on mitochondrial viability and the structural dynamics of the endoplasmic reticulum (8). The mitochondrial protein known as *BRI3BP* binds to human cervical cancer oncogene (HCCR-1), which is overexpressed in many human tumors and may function as a negative regulator of the tumor protein p53 (*p53*) gene (9). The *BRI3BP* gene, also known as HCCR-binding protein 1 (HCCRBP-1), is a human cervical cancer oncogene (*HCCR*) that regulates p53. *HCCRBP-1* works as a negative regulator of p53, promoting carcinogenesis in a range of human tissues by greatly raising protein kinase C (PKC) activity while decreasing pro-apoptotic PKC alpha and delta isoform levels. *HCCRBP-1* reduces p21 promoter activity, perhaps via p53 stabilization, resulting in defective functioning (9). The oncogene *HCCRBP-1* contributes to the development of human colorectal cancer and hepatocellular carcinoma (10,11). *HCCRBP-1* has also been reported to be overexpressed in a range of human malignancies, such as leukemia, lymphoma, and carcinomas of the kidney, ovary, and stomach (12). Therefore, BRI3BP (also known as HCCRBP-1), an oncoprotein potentially involved in the development of many human cancers, is a prospective diagnostic and therapeutic target for human malignancies.

To date, no studies have investigated the prognostic significance of BRI3BP in invasive BC and its association with aggressive tumor behavior. Thus, this study aims to elucidate the clinicopathological and prognostic significance of BRI3BP at the mRNA and protein levels using different BC cohorts. Accurate prognostication and treatment decisions for invasive BC will be aided by the identification of novel prognostic factors and therapeutic targets. We present this study in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1113/rc).

Highlight box

Key findings

- BRI3 binding protein (BRI3BP) is associated with aggressive features of tumor in breast cancer (BC).
- BRI3BP is a potential prognostic marker.

What is known and what is new?

- BC is the most cause of cancer related death in women worldwide.
- BRI3BP can predict prognosis of invasive BC.

What is the implication, and what should change now?

- More mechanistic research to investigate the potential role of BRI3BP in BC progression and metastasis is required.

Methods

mRNA analysis study cohort

An evaluation of the correlation between *BRI3BP* mRNA expression and multiple variables, including patient age, tumor grade, tumor size, lymph vascular invasion (LVI), molecular subtypes, and patient outcomes, was performed using the METABRIC (n=1,980) and TCGA (n=854) cohorts (5,6). In the METABRIC cohort, the Illumina Totalprep RNA amplification kit (Ambion, Warrington, UK) was used to generate biotin-labeled cRNA from total RNA, which was then hybridized on the Illumina Human HT-12 v3 platform to evaluate mRNA expression. For TCGA, the Genomic Data Commons Data Portal and cBioPortal websites were used to extract the cohort data. The cohort was then assessed for RNASeqV2-derived

© AME Publishing Company.

mRNA expression data (13,14).

BRI3BP protein expression study cohort

The tissue samples used in the study comprised stored retrospective tissue samples that were obtained from 100 Saudi women with invasive BC. These patients attended King Abdulaziz Specialist Hospital (KASH). Formalin-fixed paraffin-embedded (FFPE) tissue blocks with sufficient tumor tissue were used as samples in this study. The histological grade, LVI status, tumor size, lymph node status, and age at diagnosis were all recorded as part of each patient's clinicopathological profile. For this cohort, information on the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67 was available. Immunohistochemistry (IHC) was used to determine ER/PR status, with tumors classed as ER^+/PR^+ if the staining intensity was more than 1%. Furthermore, the tumor was classified as HER-2-positive if it scored 3+ via IHC or if the score was 2+ and fluorescence in situ hybridization (FISH) analysis demonstrated amplification of the *HER2* gene (15). IHC profiles were used to characterize the BC molecular subtypes based on St. Gallen subtypes: triple-negative BC (ER^-, PR^-, and $HER2^-$), luminal A (ER^+ and/or $PR^+/HER2^-$; Ki67 <20%), luminal B (ER^+ and or $PR^+/HER2^-$; Ki67 ≥20%), and $HER2^-$ enriched ($HER2^+$ regardless of ER status) (15). Outcome data were obtained, including overall survival, defined as the time from the date of diagnosis or start of treatment to the time of death. The treatment of the patients in this cohort was based on the National Comprehensive Cancer Network (NCCN) guidelines (16).

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Directorate of Health Affairs, Taif, Saudi Arabia, (Research and Studies Department) (IRB registration No. HAP-02-T-067; approval No. 838). Informed consent was obtained from all individuals prior to surgery to use their tissue materials in research.

FFPE and BRI3BP IHC staining

For IHC staining, 4-µm tissue sections were obtained using a rotary microtome (Histo-Line Laboratories, Minux® S700, Texas, USA) from invasive BC tissues and then placed on positively charged microscope slides. Sections were rehydrated in alcohols of descending grade (Fisher Scientific, E/0665DF, Leicestershire, UK) from 100% to distilled water (dH_2O) after being dewaxed in xylene (Fisher Scientific, X/2050). To inhibit endogenous peroxidase, slices were treated with a 100% methanol (Fisher Scientific, M/4056)/0.9% hydrogen peroxide solution (H_2O_2; Fisher Scientific, H/1750). As recommended by the antibody manufacturer, antigen retrieval was performed using microwave energy (citrate buffer pH 6 at 1,000 W for 20 min). Sections were then incubated in a blocking solution containing 2% (w/v) bovine serum albumin (BSA; Sigma, A8022, Gillingham, UK) in phosphate-buffered saline (PBS). The primary rabbit polyclonal anti-BRI3BP antibody (NBP188564, novusbio, Abingdon, UK) was diluted at a 1:10 ratio in the blocking buffer, followed by a 1 h incubation at room temperature (RT). Sections were washed with PBS, then treated for 40 min at RT with a 1:200 dilution of biotinylated anti-mouse secondary antibody (Vector Laboratories, PK-6102, Oxfordshire, UK) in 2% BSA. Excess antibody was washed with PBS, then incubated with avidin-biotin complex (ABC; Vector Laboratories, PK-6100) for 30 min at RT. After washing with PBS, sections were treated with diaminobenzidine (DAB; Vector Laboratories, SK-4100). After washing the slides in dH_2O, Mayers hematoxylin (Sigma, MHS16) was used to counterstain. After washing with dH_2O, sections were immersed in several degrees of ethanol, followed by xylene, and then mounted in dibutylphthalate polystyrene xylene (DPX) (Sigma, 06522). Negative and positive controls were included in the IHC experiment. For the negative control, the primary antibody was omitted from the tissue section. Colon cancer was used as positive control as recommended by the antibody manufacturer (*Figure 1A,1B*).

BRI3BP protein expression assessment

Tissue-stained sections were observed at ×40 microscopic magnification via light microscopy (Leica Microsystems, Lecia DMI 3000B, Wetzlar, Germany). The modified histochemical score (H-score) was used to assess BRI3BP cytoplasm expression in a semi-quantitative manner. During this assessment, the staining intensity was multiplied by the percentage of positive cells in each tissue. This resulted in a score ranging from 0 to 300 (17). Intensity was assessed using negative, weak, moderate, and strong scores (corresponding to a score index of 0 to 3). The percentage of positive cells for each intensity was subjectively assessed. Non-representative cores, such as those in invasive tumors with less than 15% core surface area and folded tissue during staining and processing, were excluded from the

© AME Publishing Company.

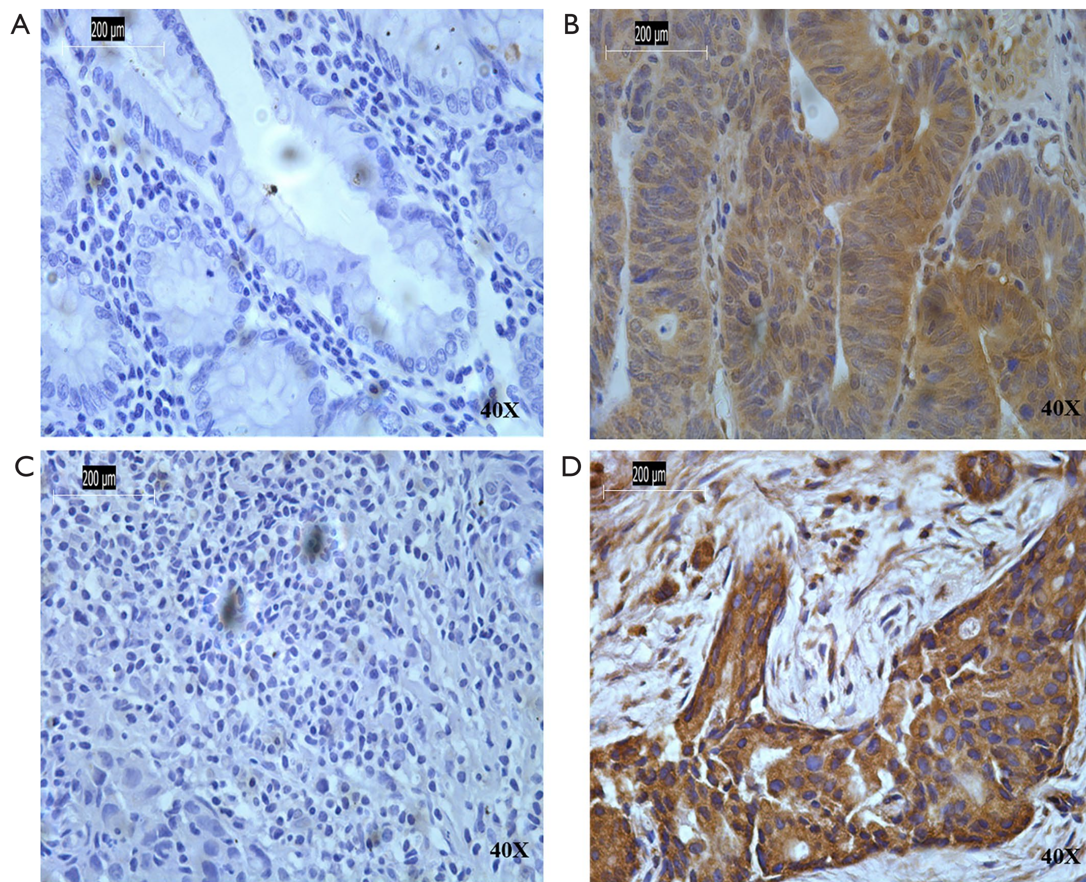

Figure 1 Cytoplasmic expression of BRI3BP proteins in invasive breast cancer. (A) Negative control of colon tissue by removing BRI3BP antibody in IHC, (B) positive control of colon tissue stained by BRI3BP in IHC, (C) BRI3BP-negative IHC expression, (D) BRI3BP-positive IHC expression. Magnification: ×40, scale bars: 200 µm. BRI3BP, BRI3 binding protein; IHC, immunohistochemistry.

scoring. A professional pathologist, working with the principal researcher, scored IHC stained slides in a blinded and individual manner for at least 20% of the cohort under evaluation. In case of a lower scoring concordance, the slides were re-scored, and the disparities in the scores were discussed by the primary researcher and a consultant pathologist. A strong concordance was found between the scorers regarding BRI3BP and immunoscoring [interclass correlation coefficient (ICC) =0.90, P<0.001]. BRI3BP protein expression data did not follow a normal distribution; hence, the median (145 H-score) was used to determine BRI3BP positivity.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) Version 24.0 (SPSS, Chicago, IL, USA). The ICC test was performed to determine the concordance rate of the BRI3BP score between the two observers. In the METABRIC cohort, data on *BRI3BP* mRNA expression were normally distributed and classified as low or high expression using a cut-off generated from the mean. The data on *BRI3BP* mRNA expression in the TCGA cohort were right-skewed and categorized using a median-generated cutoff. The correlation between BRI3BP and other clinicopathological characteristics in both cohorts was investigated using the Chi-square test. Univariate survival analysis was performed using the log-rank test and Kaplan-Meier curves. The Cox regression model was utilized for multivariate analysis. Statistical significance was determined for all tests with a two-tailed P value <0.05.

© AME Publishing Company.

Results

BRI3BP mRNA expression in BC

High *BRI3BP* mRNA expression was observed in 961/1,980 (49%) METABRIC BC cases and 427/854 (50%) TCGA cohort cases. In both cohorts, a significant correlation was observed between high *BRI3BP* mRNA expression and large tumor size (P<0.001 in METABRIC and P=0.001 in TCGA), high tumor grade (P=0.03 in METABRIC and P<0.001 in TCGA), and LVI positivity (P=0.02 in METABRIC and P<0.001 in TCGA). In the METABRIC cohort, a high *BRI3BP* mRNA level was significantly associated with ER^+ (P<0.001) and PR^+ (P=0.008). In TCGA, a high *BRI3BP* mRNA level was associated with HER2 positivity (P<0.001).

In the intrinsic (PAM50) subtypes, *BRI3BP* mRNA showed high expression with luminal B, HER2-enriched, luminal A, basal-like, and normal-like subtypes in descending order (P<0.001; *Table 1*).

BRI3BP protein expression in BC

BRI3BP protein expression was observed in the cytoplasm of invasive BC cells, with expression varying from absent to strong (*Figure 1C,1D*). A high BRI3BP protein level (H-score >145) was observed in 50/100 (50%) invasive BC cases. The correlation between BRI3BP protein expression and clinicopathological parameters in the KASH cohort was investigated. There was a significant correlation between BRI3BP protein expression and high tumor grade (P<0.001). High BRI3BP protein expression was also associated with ER^- (P=0.003), PR^- (P=0.002), and high Ki67 (P=0.004). Investigation of BRI3BP protein expression in the IHC molecular subtypes revealed high expression in triple-negative, $HER2^+$, ER^+/HER2-high proliferation, and ER^+/HER2-low proliferation variants in descending order (P=0.001; *Table 2*).

Association between BRI3BP expression and patient outcomes

In the METABRIC cohort, survival analyses of *BRI3BP* mRNA revealed that a high *BRI3BP* mRNA level was significantly associated with poor outcomes (P=0.001; *Figure 2A*). In the TCGA cohort, high *BRI3BP* mRNA expression was not significantly associated with patient outcomes (P=0.43; *Figure 2B*). At the protein level, in the univariate analysis, there was a significant association between the expression of BRI3BP and patient outcomes (P=0.01; *Figure 2C*). Cox regression analysis of the KASH cohort revealed that high expression of BRI3BP was a significant predictor of shorter survival regardless of lymph node status, tumor size, and tumor grade [hazard ratio (HR) =1.266; 95% confidence interval (CI): 1.051–1.526; P=0.01; *Table 3*].

Discussion

BC is a major health burden and the most commonly diagnosed invasive cancer among women worldwide (18). In Saudi Arabia, BC is the most diagnosed cancer and the second-leading cause of mortality (19). Therefore, it is essential to improve upon the existing options for invasive BC prevention, diagnosis, prognosis, and treatment to improve patient outcomes and quality of life. This endeavor is challenging because BC is a heterogeneous condition involving a wide variety of morphological and molecular entities, and each BC case has a unique molecular architecture, with inter-tumor or even intra-tumor heterogeneity (20,21).

In clinical practice, prognostic or predictive criteria, such as tumor size, tumor stage, histological grade, LVI, and hormone receptor status, are frequently utilized to predict outcomes and guide the selection of systemic therapy (22). Thus, identifying more prognostic and predictive biomarkers will address critical concerns about patient outcomes and aid patient management and decision-making on treatment (23). Several preclinical studies have demonstrated the potential role of BRI3BP in the progression of many cancer types, including hepatocellular carcinoma (11), leukemia, ovarian carcinoma (24), and BC (12); however, this is the first study to conduct a comprehensive analysis of the correlation between BRI3BP and clinicopathological parameters and patient outcomes at the transcriptomic and protein levels.

In a previous study using ANN on large BC cohorts, *BRI3BP* was one of the principal genes found in invasive BC samples (7). *BRI3BP* is a human gene responsible for encoding the BRI3 binding protein. BRI3BP is an oncoprotein that plays a role in human carcinogenesis and the regulation of p53. It suppresses pro-apoptotic PKC levels while considerably raising PKC activity, acting as a negative regulator of p53 and promoting carcinogenesis in a range of human tissues (9). An essential component of the *BRI3BP* oncological pathway is promoting cell proliferation followed by cell survival. This occurs via the effect of the signal produced by the PI3K/AKT or MAPK pathways,

© AME Publishing Company.

Table 1 Statistical associations between *BRI3BP* mRNA expression and clinicopathological parameters in the METABRIC (n=1,980) and TCGA (n=854) breast carcinoma datasets

Parameters	*BRI3BP* mRNA (METABRIC)[†]			*BRI3BP* mRNA (TCGA)		
	Low (n=1,018), n [%]	High (n=961), n [%]	χ^2 (P value)	Low (n=427), n [%]	High (n=427), n [%]	χ^2 (P value)
Patient age (years)			16.356 (<0.001)			
≤50	255 [60]	169 [40]		111 [48]	120 [52]	0.481
>50	763 [49]	792 [51]		316 [51]	307 [49]	0.49
Menopausal status			20.173 (<0.001)			
Pre-menopausal	266 [61]	170 [39]		Not available	Not available	Not available
Post-menopausal	748 [49]	784 [51]		Not available	Not available	
Tumor size (cm)			14.977 (<0.001)			
≤2	360 [58]	261 [42]		141 [59]	98 [41]	10.743 (0.001)
>2	650 [49]	688 [51]		286 [47]	329 [53]	
Tumor grade			6.908 (0.03)			
I	103 [61]	66 [39]		60 [67]	29 [33]	31.625 (<0.001)
II	395 [51]	375 [49]		206 [55]	169 [45]	
III	476 [50]	476 [50]		137 [39]	215 [61]	
NPI			0.878 (0.65)			Not available
Good	359 [53]	320 [47]		Not available	Not available	
Moderate	557 [51]	544 [49]		Not available	Not available	
Poor	102 [51]	97 [49]		Not available	Not available	
Lymph node stage			1.267 (0.53)	Not available		
I	527 [51]	507 [49]				
II	331 [53]	291 [47]				
III	157 [50]	159 [50]				
LVI			5.117 (0.02)			19.270 (<0.001)
Negative	525 [57]	404 [43]		310 [55]	249 [45]	
Positive	322 [51]	313 [49]		117 [40]	178 [60]	
ER			21.671 (<0.001)			0.987 (0.32)
Negative	288 [61]	186 [39]		87 [47]	98 [53]	
Positive	730 [49]	775 [51]		327 [51]	312 [49]	
PR			7.042 (0.008)			2.352 (0.13)
Negative	513 [55]	427 [45]		126 [46]	146 [54]	
Positive	505 [49]	534 [51]		284 [52]	262 [48]	
HER2			1.873 (0.17)			12.597 (<0.001)
Negative	901 [52]	831 [48]		293 [52]	274 [48]	
Positive	117 [47]	130 [53]		46 [35]	87 [65]	

Table 1 (*continued*)

© AME Publishing Company.

Table 1 (*continued*)

Parameters	*BRI3BP* mRNA (METABRIC)[†]			*BRI3BP* mRNA (TCGA)		
	Low (n=1,018), n [%]	High (n=961), n [%]	χ^2 (P value)	Low (n=427), n [%]	High (n=427), n [%]	χ^2 (P value)
PAM50 subtype			153.804 (<0.001)			Not available
Luminal A	403 [56]	314 [44]		Not available	Not available	
Luminal B	166 [34]	322 [66]		Not available	Not available	
HER2$^+$-enriched	86 [36]	154 [64]		Not available	Not available	
Basal-like	215 [65]	114 [35]		Not available	Not available	
Normal-like	146 [73]	53 [27]		Not available	Not available	

[†], some cases may not have the expression level of certain gene, which may result in a value different than 1,980. χ^2: Chi-squared. BRI3BP, BRI3 binding protein; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; TCGA, The Cancer Genome Atlas; NPI, Nottingham prognostic index; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

which cause cancer cell cycle progression and caspase-dependent apoptosis (25,26). One study investigated the role of *BRI3BP* in cancer in terms of its activity in the endoplasmic reticulum and its effect on apoptosis. Overexpression of *BRI3BP* in human embryonic kidney 293T cells, particularly when treated with the anticancer drug etoposide, resulted in increased apoptosis. In cells treated with etoposide, *BRI3BP* overexpression increased caspase-3 activity and mitochondrial cytochrome c release. This shows that *BRI3BP* may have a pro-apoptotic property and may boost the efficiency of some anticancer drugs (8).

Preclinical studies elucidated the functional roles of *BRI3BP* in some cancer types. *In vitro*, *BRI3BP* (known as *HCCRBP-3*) overexpression transformed normal cells into tumor cells in different cancer types. While p21 and bax were not properly induced in *HCCRBP-3*-transfected cells, p53 showed functional impairment. This finding is corroborated by the dose-dependent inhibition of p21 promoter activity, including p53-responsive regions, by *HCCRBP-3*. This indicates that *HCCRBP-3* inhibits p53 function, which, in turn, contributes to carcinogenesis (27). A functional experimental study demonstrated that *HCCRBP-3* increased the proliferation rate of gastric cancer cells and stimulated the development of subcutaneous tumors in nude mice (28). Furthermore, BC metastasis and development have been observed in *BRI3BP* transgenic mice via the regulation of the p53 tumor suppressor (25).

BRI3BP's interaction with tumor-infiltrating immune cells in BC remains unknown. *BRI3BP* has been found to regulate the tumor microenvironment in which immune cells like tumor-associated macrophages (TAMs), T cells, and natural killer (NK) cells exist. There is an association between an increased presentation of macrophages in the tumor microenvironment, the expression of some genes such as *BRI3BP*, and prognosis and immunotherapy (29). While this study is based on gastric cancer, the interaction of *BRI3BP* with a similar pathway in BC can be reasoned. *BRI3BP* might affect the attraction of BC-related TAMs that play a crucial role in tumor development and metastasis. The newly identified *BRI3BP* could be involved in the regulation of immune checkpoints or cytokines that prevent an effective immune response and promote an immune-suppressive tumor microenvironment, thereby inducing a more aggressive tumor phenotype (30). This indicates that high BRI3BP expression is a marker of poor prognosis in invasive BC.

BRI3BP evaluation in this study revealed that *BRI3BP* mRNA expression was associated with poor prognostic variables, including large tumor size, high tumor grade, LVI positivity, hormone receptor positivity, and HER2 positivity, demonstrating its critical role in the progression of BC. At the protein level, there was a significant association between high BRI3BP expression and high histologic grade, hormone receptor negativity, and HER2 positivity. Regarding BC molecular subtypes, high *BRI3BP* mRNA expression was significantly associated with the luminal-B and HER2-enriched variants. In agreement with the mRNA results, high BRI3BP expression at the protein level was significantly associated with HER2$^+$ molecular subtypes. The association between HER2$^+$ molecular subtype, high tumor grade, and BRI3BP mRNA and protein expression, which is unsurprising given that high grade is strongly

Table 2 Statistical associations between BRI3BP protein expression and clinicopathological factors in the King Abdulaziz Specialist Hospital breast carcinoma cohort (n=100)

Clinicopathological parameters	BRI3BP protein expression		
	Low (n=50), n [%]	High (n=50), n [%]	χ^2 (P value)
Patient age (years)			1.004 (0.32)
≤50	21 [45]	26 [55]	
>50	29 [55]	24 [45]	
Menopausal status			1.004 (0.32)
Pre-menopausal	21 [45]	26 [55]	
Post-menopausal	29 [55]	24 [45]	
Tumor size (mm)			0.158 (0.70)
≤10	15 [48]	16 [52]	
>10	15 [54]	13 [46]	
Tumor grade			41.590 (<0.001)
I	6 [75]	2 [25]	
II	39 [70]	17 [30]	
III	2 [7]	28 [93]	
Lymph-vascular invasion			2.277 (0.13)
Negative	9 [41]	13 [59]	
Positive	14 [64]	8 [36]	
ER			8.731 (0.003)
Negative	5 [23]	17 [77]	
Positive	45 [58]	32 [42]	
PR			9.400 (0.002)
Negative	6 [24]	19 [76]	
Positive	44 [59]	30 [41]	
HER2			0.054 (0.82)
Negative	36 [49]	37 [51]	
Positive	13 [52]	12 [48]	
Ki67			8.113 (0.004)
Low	26 [68]	12 [32]	
High	22 [39]	35 [61]	
Immunohistochemistry subtype			15.489 (0.001)
$ER^+/HER2^-$ low proliferation	28 [72]	11 [28]	
$ER^+/HER2^-$ high proliferation	16 [44]	20 [56]	
Triple-negative	2 [14]	12 [86]	
$HER2^+$	3 [38]	5 [62]	

χ^2: Chi-squared. BRI3BP, BRI3 binding protein; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

© AME Publishing Company.

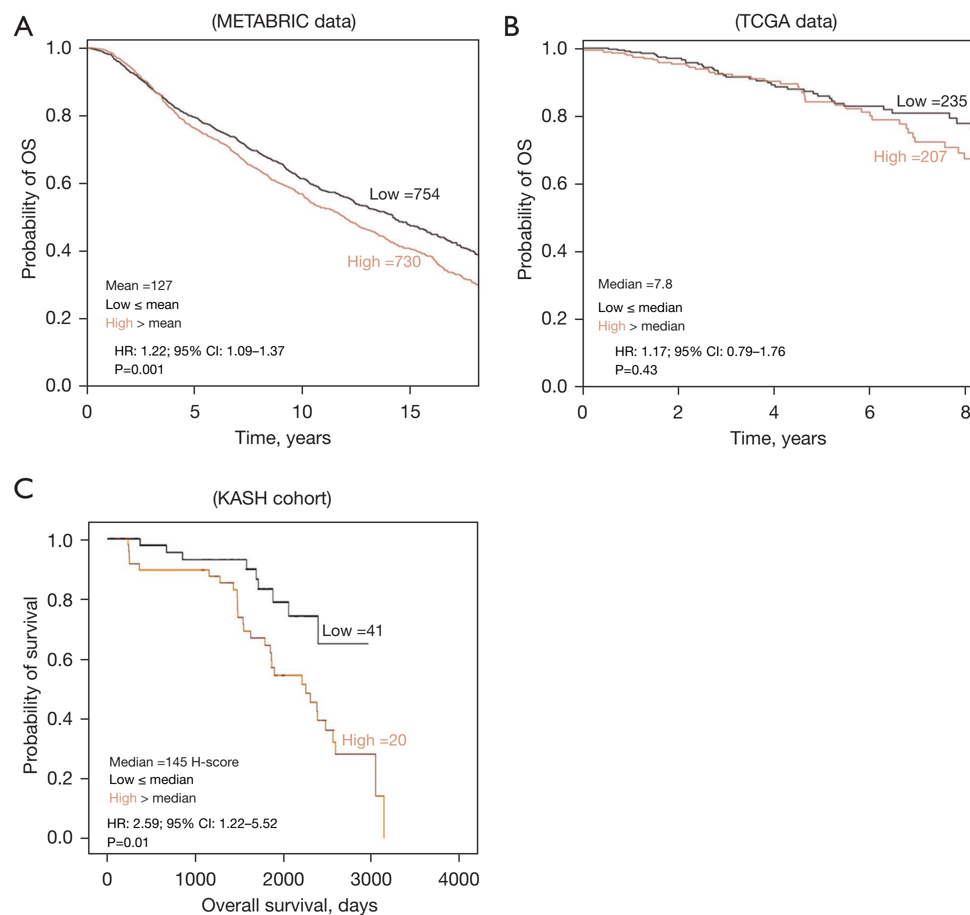

Figure 2 Kaplan-Meier survival plots showing the association between BRI3BP expression and overall survival in (A) the whole METABRIC cohort, (B) the whole TCGA cohort, and (C) the whole KASH cohort. METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; HR, hazard ratio; CI, confidence interval; OS, overall survival; TCGA, The Cancer Genome Atlas; KASH, King Abdulaziz Specialist Hospital; H-score, histochemical score; BRI3BP, BRI3 binding protein.

associated with HER2 positivity (31), refers to advanced tumors and may predict poor prognosis and outcomes. HER2 overexpression promotes cell proliferation by suppressing apoptosis, which leads to malignant tumors (31). A significant association between high BRI3BP expression at the proteomic level and triple-negative BC subtype and high Ki67 levels was also present. These findings align with previous studies reporting that the HER2-positive and triple-negative subtypes are highly proliferative tumors (32). They also revealed that high expression of the protein is associated with aggressive features of BC. Compared to the primary tumor, metastatic BC is frequently associated with aggressive prognosis groups, such as the $HER2^+$ and triple-negative molecular subtypes. A study revealed that triple-negative and HER2-positive tumors are commonly associated with brain metastasis in BC (33,34). HER2-positive and triple-negative tumors are more likely to show changes in the epidermal growth factor receptor and HER2-associated signaling pathways, which are linked to brain metastasis development and a worse prognosis in BC (35). These findings suggest that high expression of BRI3BP may lead to metastatic disease in BC as high BRI3BP expression is associated with these BC molecular subtypes.

Additionally, in the METABRIC cohort, high *BRI3BP* mRNA expression showed a significant association with poor outcomes. Investigation at the protein level also revealed a significant association between BRI3BP and short survival. Furthermore, high BRI3BP expression had a prognostic value independent of other clinical characteristics. However, the expression of *BRI3BP* mRNA in the TCGA cohort was not significantly associated with overall survival in patients with invasive BC. Many studies

© AME Publishing Company.

Table 3 Multivariate Cox regression analysis for predictors of overall survival and BRI3BP mRNA expression in the METABRIC and TCGA cohorts and protein expression in the KASH cohort

Parameters	HR	95% CI		P value
		Lower	Upper	
METABRIC cohort (mRNA)				
BRI3BP mRNA expression	1.048	0.795	1.382	0.74
Tumor size	1.345	0.999	1.810	0.051
Lymph node	1.869	1.525	2.289	<0.001
Tumor grade	2.164	1.666	2.810	<0.001
TCGA cohort (mRNA)				
BRI3BP mRNA expression	0.793	0.507	1.239	0.31
Tumor size	1.522	0.873	2.654	0.14
Lymph node	1.688	1.060	2.685	0.02
Tumor grade	1.369	0.971	1.931	0.07
KASH cohort (protein)				
BRI3BP protein expression	1.266	1.051	1.526	0.01
Tumor size	1.564	1.221	1.686	<0.001
Lymph node	2.200	1.815	2.667	<0.001
Tumor grade	1.435	1.221	1.686	<0.001

BRI3BP, BRI3 binding protein; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; TCGA, The Cancer Genome Atlas; KASH, King Abdulaziz Specialist Hospital; HR, hazard ratio; CI, confidence interval.

describe factors that might explain the differences between mRNA and protein levels. One of these factors is the cellular steady state, which describes the average protein and/or mRNA level in a cell that remains largely constant over time (36). Since mRNA levels primarily control protein levels, there will be differences in cellular mRNA and protein levels if cells are undergoing long-term dynamic processes such as continuous differentiation (36). Another factor related to the variation between mRNA and protein levels is the possible delay during state transition in the synthesis of mRNA and protein; stimulated transcription does not instantly result in higher protein levels due to the time required for mRNA maturation, export, and translation. Other factors that potentially contribute to the discrepancy between the expression of *BRI3BP* and patient outcomes at the mRNA level include the methodology utilized for measuring and statistically analyzing gene expression in the METABRIC and TCGA cohorts (37).

All these findings suggest that BRI3BP may play a critical role in the development of BC. The findings, which partly corroborate preceding investigations, suggest that BRI3BP associated with aggressive clinicopathological parameters in invasive BC could be a prognostic and predictive factor for BC. Although the pathways involved in *BRI3BP* gene regulation in tumor cells are under discussion and a detailed framework has not been elucidated, several pathways are suggested to be involved. These constitute transcriptional control, post-transcriptional control, and epigenetic control. BRI3BP may be regulated by long non-coding RNAs (lncRNAs). lncRNAs have been indicated to be involved in the control of gene expression, whether oncogenes or tumor suppressor genes (38). They do so by either modulating the *BRI3BP* gene's promoter activity in tumor cells or interacting with other signaling pathways or molecules that influence promoter activity (39). However, more comprehensive mechanistic research to thoroughly investigate the potential role of *BRI3BP* in BC progression and metastasis is warranted. Although the findings of the current study are remarkable, it has limitations, including the limited number of patient samples used for protein expression analysis and the limited number of studies available for comparison of the results. On the other hand,

this study was the first to examine the clinicopathological and prognostic importance of BRI3BP in BC and is thus a valuable addition to the field.

Conclusions

In conclusion, this study indicates that a high level of BRI3BP expression in both the transcriptome and the proteome is associated with aggressive clinicopathological characteristics of BC and poor survival outcomes. Thus, BRI3BP has the potential to serve as both a prognostic marker and a therapeutic target.

Acknowledgments

The authors would like to acknowledge Deanship of Graduate Studies and Scientific Research, Taif University (project No. 202314), for funding this work.
Funding: This research was supported by Taif University, Saudi Arabia (project No. 202314).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1113/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1113/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1113/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1113/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Institutional Review Board (Directorate of Health Affairs) (IRB registration No. HAP-02-T-067; approval No. 838). All samples collected from King Abdulaziz Specialist Hospital used in this study were pseudonymized. Informed consent was obtained from all individuals prior to surgery to use their tissue materials in research.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Barzaman K, Karami J, Zarei Z, et al. Breast cancer: Biology, biomarkers, and treatments. Int Immunopharmacol 2020;84:106535.
2. Aljondi R, Alghamdi SS, Tajaldeen A, et al. Application of Artificial Intelligence in the Mammographic Detection of Breast Cancer in Saudi Arabian Women. Appl Sci 2023;13:12087.
3. Cedolini C, Bertozzi S, Londero AP, et al. Type of breast cancer diagnosis, screening, and survival. Clin Breast Cancer 2014;14:235-40.
4. Marshall DC, Webb TE, Hall RA, et al. Trends in UK regional cancer mortality 1991-2007. Br J Cancer 2016;114:340-7.
5. Ciriello G, Gatza ML, Beck AH, et al. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. Cell 2015;163:506-19.
6. Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486:346-52.
7. Aljohani AI, Toss MS, El-Sharawy KA, et al. Upregulation of Cyclin B2 (CCNB2) in breast cancer contributes to the development of lymphovascular invasion. Am J Cancer Res 2022;12:469-89.
8. Yamazaki T, Sasaki N, Nishi M, et al. Augmentation of drug-induced cell death by ER protein BRI3BP. Biochem Biophys Res Commun 2007;362:971-5.
9. Ha SA, Shin SM, Lee YJ, et al. HCCRBP-1 directly interacting with HCCR-1 induces tumorigenesis through P53 stabilization. Int J Cancer 2008;122:501-8.
10. Shin SM, Chung YJ, Oh ST, et al. HCCR-1-interacting molecule "deleted in polyposis 1" plays a tumor-suppressor role in colon carcinogenesis. Gastroenterology 2006;130:2074-86.

11. Yoon SK, Lim NK, Ha SA, et al. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. Cancer Res 2004;64:5434-41.
12. Ko J, Shin SM, Oh YM, et al. Transgenic mouse model for breast cancer: induction of breast cancer in novel oncogene HCCR-2 transgenic mice. Oncogene 2004;23:1950-3.
13. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
14. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
15. Kunheri B, Raj RV, Vijaykumar DK, et al. Impact of St. Gallen surrogate classification for intrinsic breast cancer sub-types on disease features, recurrence, and survival in South Indian patients. Indian J Cancer 2020;57:49-54.
16. Gradishar WJ, Moran MS, Abraham J, et al. NCCN Guidelines® Insights: Breast Cancer, Version 4.2023. J Natl Compr Canc Netw 2023;21:594-608.
17. Jeon T, Kim A, Kim C. Automated immunohistochemical assessment ability to evaluate estrogen and progesterone receptor status compared with quantitative reverse transcription-polymerase chain reaction in breast carcinoma patients. J Pathol Transl Med 2021;55:33-42.
18. Giaquinto AN, Sung H, Miller KD, et al. Breast Cancer Statistics, 2022. CA Cancer J Clin 2022;72:524-41.
19. Basudan AM. Breast Cancer Incidence Patterns in the Saudi Female Population: A 17-Year Retrospective Analysis. Medicina (Kaunas) 2022;58:1617.
20. Fumagalli C, Barberis M. Breast Cancer Heterogeneity. Diagnostics (Basel) 2021;11:1555.
21. Lindström LS, Yau C, Czene K, et al. Intratumor Heterogeneity of the Estrogen Receptor and the Long-term Risk of Fatal Breast Cancer. J Natl Cancer Inst 2018;110:726-33.
22. Tarighati E, Keivan H, Mahani H. A review of prognostic and predictive biomarkers in breast cancer. Clin Exp Med 2023;23:1-16.
23. Halabi S, Owzar K. The importance of identifying and validating prognostic factors in oncology. Semin Oncol 2010;37:e9-18.
24. Ko J, Lee YH, Hwang SY, et al. Identification and differential expression of novel human cervical cancer oncogene HCCR-2 in human cancers and its involvement in p53 stabilization. Oncogene 2003;22:4679-89.
25. Liao S, Liang L, Yue C, et al. CD38 is involved in cell energy metabolism via activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells. Int J Oncol 2020;57:338-54.
26. Liu X, Haniff HS, Childs-Disney JL, et al. Targeted Degradation of the Oncogenic MicroRNA 17-92 Cluster by Structure-Targeting Ligands. J Am Chem Soc 2020;142:6970-82.
27. Ha SA, Kim HK, Yoo JA, et al. HCCRBP-3 induces tumorigenesis through direct interaction with HCCR-1 in human cancers. Mol Carcinog 2014;53:30-7.
28. Zhu LF, Ma P, Hu YL, et al. HCCR-1 is a Novel Prognostic Indicator for Gastric Cancer and Promotes Cell Proliferation. J Cancer 2019;10:3533-42.
29. Xin H, Chen Y, Niu H, et al. Integrated Analysis Construct a Tumor-Associated Macrophage Novel Signature with Promising Implications in Predicting the Prognosis and Immunotherapeutic Response of Gastric Cancer Patients. Dig Dis Sci 2024;69:2055-73.
30. Wang M, Zhang C, Song Y, et al. Mechanism of immune evasion in breast cancer. Onco Targets Ther 2017;10:1561-73.
31. Lv Q, Meng Z, Yu Y, et al. Molecular Mechanisms and Translational Therapies for Human Epidermal Receptor 2 Positive Breast Cancer. Int J Mol Sci 2016;17:2095.
32. Dai X, Xiang L, Li T, et al. Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes. J Cancer 2016;7:1281-94.
33. Klebe M, Fremd C, Kriegsmann M, et al. Frequent Molecular Subtype Switching and Gene Expression Alterations in Lung and Pleural Metastasis From Luminal A-Type Breast Cancer. JCO Precis Oncol 2020;4:PO.19.00337.
34. Sihto H, Lundin J, Lundin M, et al. Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. Breast Cancer Res 2011;13:R87.
35. Bryan S, Witzel I, Borgmann K, et al. Molecular Mechanisms Associated with Brain Metastases in HER2-Positive and Triple Negative Breast Cancers. Cancers (Basel) 2021;13:4137.
36. Liu Y, Beyer A, Aebersold R. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell 2016;165:535-50.
37. Prabahar A, Zamora R, Barclay D, et al. Unraveling the complex relationship between mRNA and protein abundances: a machine learning-based approach for imputing protein levels from RNA-seq data. NAR Genom Bioinform 2024;6:lqae019.

© AME Publishing Company.

38. Zhang Y, Wang X, Hu C, et al. Shiny transcriptional junk: lncRNA-derived peptides in cancers and immune responses. Life Sci 2023;316:121434.
39. Li XL, Pongor L, Tang W, et al. A small protein encoded by a putative lncRNA regulates apoptosis and tumorigenicity in human colorectal cancer cells. Elife 2020;9:e53734.

Cite this article as: Aljohani AI, Aljahdali IA, Alsalmi OA, Alsuwat MA, Alsharif AA, Alzahrani KJ, Alsaleh BS, Nadheef A, Alqurashi TS. The clinicopathological significance of BRI3BP in women with invasive breast cancer. Transl Cancer Res 2024;13(12):6837-6849. doi: 10.21037/tcr-24-1113